APPENDIX 3

MATERIALS AND METHODS FOR EXPERIMENTS

Alpha (2) macroglobulin purification.

For purification of α2M, serum from mice was diluted 1:1 with 0.04M Tris pH 7.6, 0.15M NaCl and applied to a 65ml Sephacryl S 300R (SIGMA) column equilibrated and eluted with the same buffer. α2M-positive fractions were determined by a dot-blot and the buffer in the fraction was changed to a 0.01M sodium phosphate buffer pH 7.5 by use of a PD-10 column. The protein-containing fractions were applied to a Concanavalin A sepharose column. Bound protein was eluted with 0.2M methylmannose pyranoside and applied to a DEAE column equilibrated with 0.05M sodium acetate buffer. α2M was eluted in a pure form as analyzed by SDS-PAGE and immunoblotting with 0.13M sodium acetate. In some experiments, α2M was purchased from SIGMA.

Tumor rejection assays using OVA20

Ovalbumin peptide (OVA20), a 20-mer extended variant (NH_2-SGLEQLESIINFEKLTEWTS-COOH) of the K^b-binding epitope ova8 of ova was synthesized at Genemed Synthesis (San Francisco, CA). C57BL/6 mice were immunized intradermally with Protein-peptide complexes twice, at a one week interval on days -14 and -7. The immunization regimen is outlined in Figure 1 (top). The mice were challenged with 100 000 live B16-F10-OVA (ovalbumin-expressing) tumor cells one week after the last immunization. α2M or gp96 was complexed with synthetic OVA20 peptide. Groups of five mice were used for each of the protein-peptide complexes administered. As a positive, control mice were immunized with gp96-ova20 complexes. Mice were also immunized with alpha2M or gp96 without peptide or with PBS. Tumor growth (and survival) was monitored and tumors growth measured in mm^3.